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Mucosally targeted immunization

The present invention relates to an immunization method which makes it possible to induce a local immune response, in particular in the rectogenitourinary mucosal region. Another subject matter of the invention is the use of immunogens in the preparation of vaccine compositions intended to be administered according to said method.

State of the art

The main route for the transmission of the AIDS virus is the mucous membranes, in particular genital and rectal mucous membranes, indeed even buccal mucous membrane. From these mucous membranes, the virus rapidly spreads to the draining lymph nodes, before joining the peripheral blood.

As in the case of other pathogenic agents (virus, bacterium, and the like) with a mucosal gateway, the induction of immunity capable of blocking the virus at its entry into the mucous membrane or in the first stages of the spreading thereof into the lymph nodes appears to be important.

The majority of studies are generally targeted at obtaining a systemic immunity which is detectable by the titer of serum antibodies, as described by Jian-Ming X. et al. (Vaccine, 993-1000, 1996), Kim J.J. et al. (Vaccine, 879-883, 1997), Anderson et al. (The Journal of Infectious Diseases, 960-969, 1989), David D. et al. (Vaccine, 1661-1669, 1997), Raskisov et al. (US 4 368 191) and Transgène (FR 2 751 879).

The targeting of a local immunity of the mucosal type can be demonstrated by the presence of specific antibodies present in the mucous membranes or the

secretions (Thibodeau L. et al. Aids Research and Human Retrovirus, 1379, 1992; Russel M.W. et al., Reviews of Infectious Diseases, 5440-5446, 1988).

5 The studies of Lehner et al. (Nature Medicine, 767-775, 1996), carried out on the rhesus macaque, have shown that it is possible to induce a local immunity with respect to the SIV virus by carrying out an extremely deep subcutaneous injection in the pelvic region in the
10 vicinity of the iliac lymph nodes. This immunization is reflected by the induction of antibodies of IgA and IgG type in the rectal and urinary fluids and in the serum. However, such an immunization method is not applicable to man.

15 Letchworth G.J. et al. (US 5 462 734) teach that an intramuscular injection (without specifying the injection site) of a glycoprotein induces solely a systemic response and the booster applied in the mucous
20 membranes then makes it possible to obtain a local mucosal immunity. It thus seems that the intramuscular injection does not make possible, by itself alone, the targeting of a local response.

25 Gaffar A. et al. (US 3 931 398) teach that an injection of a vaccine in the oral cavity would make it possible to induce a local immunity. No measurement of the antibodies is reported. It thus seems that the proximity of the injection site to the site of
30 targeting of a local immunity is essential, as also reported by Letchworth G.J. et al. above.

There thus currently exists no mucosal immunization method targeted at the rectogenitourinary region which
35 is truly effective and usable in the current practice of human or veterinary medicine. Furthermore, there was nothing to allow it to be supposed that an injection at sites distant from the mucous membranes could bring about a targeted local response.

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Summary of the invention

5 In order to meet the need to have available technical
solutions for effectively and easily inducing a local
immunity, the present invention is thus targeted at the
use of an immunogen specific for a pathogenic agent
having a gateway in a mucous membrane for the
10 production of an immunogenic composition intended to be
administered to man by the parenteral route at the
surface of a part of the body distinct and distant from
said mucous membrane, so as to directly develop a local
response in respect of IgA, IgG and/or IgM antibodies
and of B cells which secrete said antibodies in said
15 mucous membrane and the lymph nodes which drain it.

A particular objective of the invention relates to the
targeting of the rectal, genital and/or urinary mucous
membranes by administration by the parenteral route in
20 the thigh. In the face of this report, it is now thus
possible to look for multiple sites for injections,
distinct from the mucous membranes, which will make
possible, directly, the induction of a local response
in these mucous membranes, in particular in respect of
25 antibodies and of B cells which secrete antibodies
found in the mucous membranes and the lymph nodes which
drain them.

Another particular objective of the invention is to
30 provide such an immunization route in developing a
local immunity against the AIDS (HIV) virus.

Another objective of the invention is to simultaneously
induce a systemic immunity, in particular a humoral
35 and/or cellular immunity, detectable in the peripheral
blood.

Details of the invention

5 A subject matter of the present invention is thus the development of a local response, in particular in the rectogenitourinary mucous membrane and the lymph nodes which drain it, by parenteral injection of an immunogenic composition in a part of the body distinct from the mucous membrane, such as the thigh. The parenteral administration in the thigh and in the 10 higher neighboring regions, in particular the groin, makes it possible to target the iliac and inguinal lymph nodes. The intramuscular route in one or both lower limbs, in particular in the quadriceps, especially in the right anterior muscle, is preferred. 15 Such an immunization route proves to be capable of inducing locally, in this mucous membrane, on the one hand, the production of immunoglobulins in the secretions and, on the other hand, regionally, in the lymph nodes which drain this mucous membrane, the 20 production of B cells which secrete antibodies, while inducing a systemic immunity. This immunity is capable of inducing protection against the entry and the spreading of the pathogen under consideration from this rectogenitourinary mucous membrane region.

25 The invention applies both to the field of prophylaxis (e.g. vaccines) and to the field of active immunotherapy. The term "immunogenic composition" thus covers compositions with a prophylactic purpose, in 30 particular vaccines, and compositions with a curative purpose, in which compositions the immunogen is of antigen type.

35 A first specific subject matter of the invention is thus the use of an immunogen specific for a pathogenic agent having a gateway in the rectogenitourinary mucous membrane region for the production of an immunogenic composition intended to be administered to man by the parenteral route in the thigh, preferably by the

intramuscular route, in particular in the quadriceps, especially in the right anterior muscle (the parenteral administration in a muscle of the thigh will preferably take place in the muscle of each right and left lower limb), so as to develop a local response in respect of IgA antibodies, indeed even also of IgG and/or IgM antibodies, and of B cells which secrete IgG, IgM and/or IgA, in respectively, on the one hand, the rectogenitourinary mucous membranes and their secretions and, on the other hand, the lymph nodes which drain this mucous membrane, in particular inguinal lymph nodes and external and internal iliac lymph nodes. This is because the parenteral injection and in particular the intramuscular injection in the thigh turns out to make possible the recruitment of B cells which produce IgG, IgM and/or IgA antibodies in the lymph nodes which drain the rectogenitourinary mucous membrane region.

The production of IgA and IgG antibodies of plasma or secretory origin and the local recruitment of B cells which secrete IgA and IgG are thus achieved, as was observed in the tests reported in points III and V of the examples.

Mention will very particularly be made, among the pathogenic agents to which the invention can be applied, of: the HIV virus, Herpes viruses, e.g. the Herpes simplex virus, in particular of type 2, Candida species, Chlamydia species, the human papillomavirus, genital mycoplasmas, Treponema pallidum, papovaviruses, e.g. Condyloma accuminatum, and gonococcal infections.

It should be noted that this method of administration is not limited to inducing a local response and can also make it possible to induce, at the same time, a systemic response, the two actions combining together and complementing one another, indeed even reinforcing one another, in a particularly advantageous way.

Consequently, the use in accordance with the invention is also targeted at developing, in addition to a local response according to the invention, a systemic response of IgG and/or IgM and optionally IgA type (antibodies and secretory B cells).

Without it being necessary to specify it each time, it is obvious that, when speaking of a response in respect of IgG, IgM or IgA, as antibodies and secretory B cells, it is a response specific to the immunogen used. For other immunogens, the response may be in addition or instead a local cellular immune response (cytotoxic T lymphocytes, TH1 and TH2 response, optionally suppressor response).

Another subject matter of the invention is the method of immunization against pathogenic agents, such as those described above, which consists in administering, by any means known per se, the appropriate immunogenic composition by the parenteral route, in particular the intramuscular route, in the thigh of one or both lower limbs, preferably quadriceps, in particular right anterior muscle. Without it being necessary to restate it each time, the method of immunization can have each of the characteristics, alone or in combination, set out here in the context of the use.

In the context of the use and of the method of immunization, it may be further specified that the invention applies to all known types of immunogenic composition and in particular known vaccines, whether they are of conventional type or of recombinant type. As is known per se, the compositions, e.g. vaccines, of conventional type include attenuated or inactivated live whole compositions, e.g. vaccines, or subunits (proteins or peptides). They can be adjuvanted or non-adjuvanted and be presented in the combined form grouping together different valences and/or different immunogenic forms with the same valency. The

recombinant compositions, e.g. vaccines, include the living vectors expressing one or more immunogens of the pathogen under consideration and the polynucleotide plasmid vectors composed of a DNA, which can, for example, be naked or included in a liposome (see, e.g., WO-A-90 11092, WO-A-93 19813, WO-A-94 21797, WO-A-95 20660), which express one or more immunogens. As regards recombinant living vectors, mention may in particular be made, as vectors, of pox viruses, such as the vaccinia virus and especially avian pox viruses (canarypox, fowlpox, pigeonpox, and the like), such as those described in Tartaglia et al., Virology, 1992, 188, 217, and adenoviruses. As regards a novel administration route, it is very obvious that the invention cannot be limited to a specific type of composition, e.g. vaccines, but can be applied to all types of immunogenic compositions, e.g. vaccines, and to all available compositions, e.g. vaccines, which can be used by the parenteral route, in particular the intramuscular route.

Likewise, the immunization protocol will depend on the type of composition or on the composition used. It will include the number of administrations generally used for a given composition, which will generally correspond to more than one administration, in particular from 2 to 4. A person skilled in the art is in any case fully able, by routine tests, to determine the optimum number of administrations (e.g. primary vaccination and booster). However, it should be noted that the injection in a site distinct from the mucous membranes can make possible, by itself alone, the targeting of a local response, without requiring a booster to be applied in the mucous membranes.

This targeted immunization can also be combined with a conventional systemic immunization by the same composition or another composition combating the same pathogen.

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It is also possible to combine with it, in the same host, an immunization protocol targeted at the buccal mucous membrane comprising the administration of an identical or different immunogenic composition, in particular of the same composition, which combats the same pathogen, targeted at inducing a local response in respect of IgG, IgM and/or IgA and of B cells which secrete IgG, IgM and/or IgA respectively in the saliva and the lymph nodes which drain the buccal mucous membrane, in particular the submaxillary lymph nodes (it can also be accompanied by a systemic reaction), preferably by sublingual injection in the floor of the mouth. The test reported in points III and IV shows that a sublingual injection is an appropriate means, other administration routes in the oral cavity remaining possible. It is, of course, possible to use other means capable of suitably activating the lymph nodes which drain the buccal mucous membrane, in particular the submaxillary lymph nodes.

Such a combination is particularly useful for preventing or treating an infection by a pathogen having both buccal and rectogenitourinary gateways. Mention may in particular be made of the HIV virus, the Herpes virus, and the like.

Consequently, according to an advantageous development of the present invention, the invention also relates to the use of an immunogenic composition intended to be administered to a host by the parenteral route in the thigh, in particular by the intramuscular route in the thigh, preferably in the quadriceps, in particular in the right anterior muscle, and, moreover, to the use of another immunogenic composition, identical to or different from the preceding one, intended to be administered to the same host by sublingual injection in the floor of the mouth.

The use in accordance with the invention and the method of immunization in accordance with the invention have a preferred application in the context of vaccination against the HIV virus.

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A specific example is the use of a vaccine combining together a vector expressing HIV gp120/gp160 and the gp120/gp160 glycoprotein subunit of this same virus. A specific example is described later.

10

In the context of the present invention, the use is thus anticipated of this anti-HIV vaccine for its administration by the intramuscular route in the thigh, optionally in combination with a sublingual administration and/or a systemic administration of conventional type, e.g. by intramuscular injection in the deltoid.

15

Finally, a further subject matter of the invention is an immunogenic composition comprising at least one immunogen specific for a pathogen having a gateway in the rectogenitourinary mucous membrane region and a pharmaceutically acceptable vehicle or excipient, this vehicle or excipient or this composition resulting, in combination with the immunogen, in a local response in respect of IgG, IgM and/or IgA antibodies and of B cells which secrete IgG, IgM and/or IgA in this mucous membrane region when the composition is administered by the parenteral route in the thigh, in particular by the intramuscular route. In the context of this immunogenic composition, the characteristics set out here with respect to the other subject matters of the invention can be taken up again, alone or in combination.

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The invention will now be described in more detail with the help of embodiments taken as nonlimiting examples. It must be clearly understood that the invention defined by the appended claims is not limited to the specific embodiments indicated in the above description

but encompasses the alternative forms thereof which do not depart either from the scope or from the spirit of the present invention.

5 Example 1 - Targeting of the lymph nodes

The targeting of the lymph nodes involved in the rectogenitourinary responses by the intramuscular route in the thigh is illustrated by experiments carried out
10 on the cynomolgus macaque: in these experiments, a 2% solution of Evans blue dye in saline solution was injected (0.5 to 1 ml) in the right anterior muscle at a site situated at a distance of one third from the groin and of two thirds from the knee.

15 Dissection of the euthanized animals 4 hours after injection of the dye allowed the lymph nodes draining the inoculation region to be located by virtue of their blue coloring: inguinal and iliac lymph nodes were thus
20 identified.

Example II - Vaccine

II-1 Recombinant live vaccine vCP205, ALVAC-HIV:

25 vCP205 is an ALVAC canarypox virus, the construction of which is disclosed in Example 14 of WO-A-95 27507, to which a person skilled in the art may refer. It is capable of expressing the env, gag and pro genes of the
30 HIV-1 virus. These genes are inserted in the C3 locus and are regulated by the H6 and I3L promoters of the vaccinia virus.

A pHIV32 plasmid comprising the expression cassettes
35 for the gene of the env gp120 MN glycoprotein (plus the transmembrane part of gp41 LAI) and the genes of the LAI strain coding for gag and for the pro protease was used as donor plasmid in an in vivo recombination procedure for producing vCP205. These cassettes were

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Figure 1

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II-2 gp160MN/LAI-2 subunit vaccine

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5 detrimentally affecting the amino acid sequence. An
SmaI restriction site was also created immediately
upstream of the sequence coding for the cleavage sites
between gp120 and gp41, without detrimentally affecting
10 the amino acid sequence. The two cleavage sites at
position 507-516 (numbering of the amino acids
according to Myers et al. in: Human retroviruses and
AIDS (1994), Los Alamos National Lab. (USA)) were
mutated (original sequence: KRR...REKR mutated to
15 QNH...QEHN). The sequence coding for the transmembrane
hydrophobic peptide IFIMIVGGLVGLRIVFAVLSIV (amino acids
689-710 according to Myers et al. above) was deleted. A
stop codon was introduced instead of the second codon E
of the sequence coding for PEGIEE (amino acids 735-740
20 according to Myers et al.), that is to say the 29th
amino acid of the intracytoplasmic domain.

25 The plasmid in which the LAI sequence was inserted
between the homologous regions of the TK gene of the
vaccinia virus was cleaved by SphI and SmaI and then
linked to the gp120 MN sequence. The VVTG9150 was
subsequently constructed by conventional homologous
recombination and propagated in order to ensure the
expression of the gp160 according to the method
25 generally used for vCP205 on BHK21 cells. The gp160 was
subsequently purified by immunoaffinity chromatography.

Example III - Test 1

30 Two female rhesus macaques (P9224 and P9225), already
immunized by the intramuscular route (in the left or
right thighs, alternately) twice with $10^{6.5}$ CCID₅₀ of
clarified ALVAC-HIV (vCP205) and then three times with
100 µg of gp160 MN/LAI-2 adjuvanted with OspA (outer
35 surface protein A of *Borrelia burgdorferi*) and aluminum
hydroxide, were inoculated twice at intervals of 1
month in the floor of the mouth (sublingual) with a
mixture comprising 10^6 CCID₅₀ of clarified vCP205 and
100 µg of gp160 MN/LAI-2. The saliva, the urine, the

vaginal and rectal secretions, and the serum were analyzed by ELISA in order to detect the presence of anti-gp160 and anti-CPpp (which combat the canarypox virus itself) IgA and IgG.

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One of the two monkeys (P9225) received an additional injection of the same mixture (vCP205 + gp160 MN/LAI-2) in the floor of the mouth and the top of the right thigh, three months after the final injection. The lymphocytes of the peripheral blood and of some lymph nodes (submaxillary, axillary, inguinal and iliac) lymph nodes were analyzed by ELISPOT for the detection of B cells which produce IgA and IgG antibodies specific for gp160 and CPpp.

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The appearance of anti-gp160 and anti-CPpp IgA in the mouthwash from the macaque P9224 and of anti-gp160 IgA in the vaginal wash from the macaque P9224 could be shown. The specific anti-gp160 IgG responses appeared from the first injection in the majority of the secretions tested and were maintained throughout the study.

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Furthermore, the sera of the two macaques showed a significant increase in the IgAs and IgGs specific for gp160 and CPpp.

25

Finally, a preferential induction of B cells which secrete anti-gp160 and anti-CPpp IgA⁺ and IgG⁺ antibodies in the lymph nodes targeted by the immunizations, namely the submaxillary lymph nodes and the right inguinal and iliac lymph nodes, was demonstrated by ELISPOT in the monkey P9225. These cells were also present in the peripheral blood but at a lower frequency.

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To conclude, this test showed the possibility of inducing a local and systemic anti-HIV-1 antibody response in the rhesus monkey after immunization close

to lymph nodes which drain the buccal and rectogenito-urinary mucous membranes.

Example IV - Test 2

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The vaccine is a mixture comprising $10^{6.3}$ CCID₅₀ of vCP205 and 100 µg of gp160 subunit. An ALVAC vector not comprising any HIV sequence was also used, as control.

- 10 - Group 1: 4 monkeys (rhesus macaques) received, on 4 occasions at an interval of 1 month, an injection of the vaccinal mixture by the sublingual route in the floor of the mouth; volume for volume mixture of $10^{6.3}$ CCID₅₀/ml vCP205 and of 400 µg/ml gp160; 0.25 ml on the right and 0.25 ml on the left.
- 15 - Group 2: 4 monkeys (rhesus macaques) received, on 4 occasions at an interval of 1 month, an injection of the vaccinal mixture by the intramuscular route in the thigh (perpendicular to and in the right anterior muscle): volume for volume mixture of $10^{6.6}$ CCID₅₀/ml vCP205 and of 200 µg/ml gp160; 0.5 ml on the right and 0.5 ml on the left.
- 20 - Group 3 (controls): 3 monkeys (rhesus macaques) received, on 4 occasions at an interval of 1 month, an injection of the ALVAC vector by the intramuscular route in the thigh; $10^{6.3}$ CCID₅₀/ml ALVAC (CPpp); 0.5 ml on the right and 0.5 ml on the left.
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The number of B lymphocytes which secrete total IgG's specific for the gp160 (resulting from the two types of vaccine) and specific for the control ALVAC vector per 35 10^6 mononucleated cells was measured by sampling from each group of macaques. The calculated mean and the calculated standard deviation were rounded off to the nearest unit.

- The right and left lymph nodes of each category (sub-maxillary, axillary, inguinal, internal iliac and external iliac) were removed after sacrificing the animal, milled (the right and left submaxillary lymph nodes were pooled) and then subjected to analysis of the antibody-producing cells by the ELISPOT technique (adapted from Erikson K. et al., Journal of Immunological Methods, 153, 107-113, 1992).
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- 10 A systemic response in respect of IgG antibodies was also observed.

The results of counting the IgG-secreting B lymphocytes are combined in the following table:

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			IgG ⁺ B lymphocytes per 10 ⁶ mononucleated cells		
Group (No. of monkeys/ group)	Immunization: Immunogenic route	Sample (sacrifice at W14, after 4 injections)	Totals	gp160	CPpp
1 (n=4)	Sublingual injection ALVAC-HIV (vCP205) + gp160 MM/LAI-2	Blood	167±48	1±0	1±1
		Submaxillary lymph nodes	1168±271	190±127	141±63
		Axillary lymph nodes	430±311	0±0	2±2
		Internal iliac lymph nodes	662±345	2±1	2±1
		External iliac lymph nodes	996±508	1±1	3±3
		Inguinal lymph nodes	320±83	1±1	2±1
2 (n=4)	IM injection (thigh) ALVAC-HIV (vCP205) + gp160 MM/LAI-2	Blood	152±40	1±0	2±0
		Submaxillary lymph nodes	575±156	2±1	2±1
		Axillary lymph nodes	1251±393	2±0	2±1
		Internal iliac lymph nodes	657±188	9±4	4±6
		External iliac lymph nodes	752±179	277±146	226±175
		Inguinal lymph nodes	817±199	62±88	11±13
3 (n=3)	IM injection (thigh) ALVAC vector (CPpp)	Blood	173±46	0±0	3±1
		Submaxillary lymph nodes	540±148	0±0	1±1
		Axillary lymph nodes	624±132	0±0	1±0
		Internal iliac lymph nodes	612±67	0±0	4±4
		External iliac lymph nodes	700±162	0±0	248±202
		Inguinal lymph nodes	531±83	0±0	210±80

Example V - Test 3

The vaccine is composed of two products injected separately, first of all $10^{6.5}$ CCID₅₀ of vCP205 and then 50 µg of gp160 MN/LAI subunit adjuvanted with a conventional adjuvant.

2 female rhesus macaques (#P952 and #P9315) received:

- on 2 occasions at weeks 0 and 4, an injection of vCP205 by the intramuscular route in the thigh (perpendicular to and in the right anterior muscle), on the right and then on the left alternately, at a dose of $10^{6.5}$ CCID₅₀ in a volume of 1 ml,
- on 5 occasions at weeks 8, 12, 25, 99 (for P952) or 100 (for P9315) and 103 (for P952) or 104 (for P9315), an injection of adjuvanted gp160 MN/LAI by the intramuscular route in the thigh (perpendicular to and in the right anterior muscle), on the right and on the left, at a dose of 50 µg (+ 500 µg of adjuvant) in a volume of 1 ml (0.5 ml in each thigh).

The number of B lymphocytes which secrete IgG and IgA specific for the gp160 per 10^6 mononucleated cells was measured in each sample (lymph nodes and blood) from the two macaques. The mean and the standard deviation of the counts carried out in triplicate for each sample are represented. The right and left lymph nodes of each category (submaxillary, axillary, inguinal, internal iliac and external iliac) were removed after sacrifice of the animal and milled, and the right and left lymph nodes of each category were pooled and then subjected to analysis of the antibody-producing cells by the ELISPOT technique (adapted from Eriksson K. et al., Journal of Immunological Methods, 153, 107-113, 1992).

The anti-gp160 IgA and IgG antibodies in the serum and the mucous secretions (urine, vaginal washes and rectal

washes) from the two macaques were also assayed by the ELISA technique. The samples were taken before the two late boosters (W99) and then after immunization, immediately before the sacrifice of the animal (W104 or W105). The results are expressed, for each secretion, in the form of the ratio of the specific anti-gp160 IgA or IgG activity measured at the time of the sacrifice to that evaluated before the two late boosters (W99). It was considered that a secretion was significantly positive in respect of specific IgAs or IgGs if this ratio was greater than 3 (the specific activity of a secretion corresponds to the titer in respect of specific IgAs or IgGs (in this instance for gp160) divided by the titer in respect of total IgAs or IgGs).

Furthermore, the local or plasma origin of the specific IgAs or IgGs in a secretion was evaluated according to two different techniques:

1. Measurement of the relative excretion coefficient of the Ig(A or G)s with respect to albumin (protein of essentially plasma origin), according to the technique described by Bélec L. et al., AIDS Research and Human Retroviruses, 12, 157-167, 1996.
2. Measurement of the local production coefficient of the Ig(A or G)s, which compares the specific activity measured in the secretion with that in the serum withdrawn at the same time, according to the technique described by Van Cott T. et al., Journal of Immunology, 160, 2000-2012, 1998.

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Results with regard to the antibody-secreting cells:

Monkey	Route	Sample (sacrifice at W104 or W105 after 7 injections)	Anti-gp160 IgG' B lymphocytes per 10 ⁵ mononucleated cells	Anti-gp160 IgA' B lymphocytes per 10 ⁵ mononucleated cells
P925	IM injection (thigh) ALVAC-HIV (VCP205) then adjuvanted gp160 NW/LAI-2	Blood	2 ± 1	0 ± 0
		Submaxillary lymph nodes	1 ± 0	0 ± 0
		Axillary lymph nodes	3 ± 2	0 ± 0
		Internal iliac lymph nodes	98 ± 39	11 ± 2
		External iliac lymph nodes	552 ± 159	149 ± 31
		Inguinal lymph nodes	54 ± 8	11 ± 2
P9315		Blood	3 ± 1	3 ± 0
		Submaxillary lymph nodes	2 ± 0	0 ± 0
		Axillary lymph nodes	2 ± 1	1 ± 0
		Internal iliac lymph nodes	1057 ± 311	287 ± 35
		External iliac lymph nodes	892 ± 219	346 ± 39
		Inguinal lymph nodes	227 ± 96	123 ± 43

Results with respect to the immunoglobulins present in the secretions

Ratio of the specific anti-gp160 IgG activities at W104 or W105 versus W99		
Secretions	Monkey#	Ratio
Urine	P952	8.9
	P9315	51.3
Rectal washes	P952	3.5
	P9315	57.5
Vaginal washes	P952	6.3
	P9315	57.9

- 5 IgAs were also detected in some urinary, vaginal and rectal secretions.

Example VI

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Vaccinal preparations against Herpes simplex, a Candida, a Chlamydia, a human papillomavirus, a genital mycoplasma or Treponema pallidum were injected in the muscles of the thigh in order to stimulate and target a

- 15 local response in respect of IgA antibodies at least in the rectal, genital and/or urinary mucous membranes.